THE USE OF VAPOR FRACTOMETRY IN THE ANALYSIS OF SOME NEW ENGLAND HOPS[1]

EDWARD L. DAVIS AND RICHARD L. BURNS

In recent years a number of articles have been published on the use of chemical characteristics in taxonomic studies (e.g. Turner and Alston, 1959; Alston and Irwin, 1961). These examples have shown that chemical analysis can be extremely useful in areas where extensive morphological or taxonomic information has been accumulated. Not only may the chemical analysis "solve" certain taxonomic problems, but it may also serve to substantiate conclusions formed originally on morphological features alone and in this way give greater credence to studies which have been based heavily upon qualitative rather than quantitative observations.

We are reporting here the use of a very sensitive method of chemical analysis, vapor fractometry, as it is applied to the problem of subspecific variations in the hops plant, and its correlation with morphological studies which have been done previously. The genus *Humulus* is a small one consisting of two distinct species, one a perennial and the other an annual. Several attempts have been made to distinguish two or more separate species of the perennial hops. The difficulty of this separation, from herbarium material, has been considered in detail (Davis, 1957). Within the perennial species, *Humulus lupulus* L., it is clear that there are variants, but these are not distinct enough to justify specific rank. After a study of cultivated hops from throughout the world, and wild hops of North America, the perennial hops were divided into "complexes" which represent approximately varietal differences. The differences between these complexes were based upon cytological and morphological features. Such features as the angularity and scar occurring on the cone axis; pubescence, dentation and lobing of the leaf; venation of the cone bract; and pairing of the sex chromosomes have been used. It is still premature to

[1]This research was supported by a grant from the National Science Foundation, NSF-G18013.

attempt to delimit these complexes in a definite way. Additional studies since 1957 have shown the complexity of the situation. Neve's work on hops chromosomes (1956, and personal communications) make it necessary to withhold final judgement as to chromosome types which might fit American, Continental and English complexes of hops. Work is being done at present upon the cytological features of the hops in the American Rockies and this should lead to more positive conclusions.

A comparison of morphological features and alpha acid content among cultivated varieties suggests that there is a difference in the proportion of humulone and cohumulone between the different morphological groups. Rigby (1956) and Howard and Tatchell (1955) classified hops according to the cohumulone content of the humulone complex and made 4 groups with approximately 20%, 30%, 40% and over 40% cohumulone. Examination of morphological features of many of these varieties show those with 20-25% cohumulone to correspond very closely to the Continental complex; those with 26-35% cohumulone to the English complex, and those with cohumulone of 36% and above to the American type. Considerable caution must be used in drawing these comparisons. It is necessary to establish that these chemical differences are not due to environmental conditions. Fortunately, extensive evidence indicates that although the absolute amounts of these substances are changed by the environment, the relative proportions are not. Commercial samples of the same variety, grown in very different habitats and in different seasons have fallen into the same chemical groups (Howard and Tatchell, 1956). Additional plant by plant studies over a number of years, already in progress, will be desirable to confirm this, but present evidence favors the stability of the chemical characteristics. A more fundamental problem is related to drawing conclusions about a correlation of structural features and chemical tests based upon the kind of samples which have been examined so far. Neve and Weston (1958) have very clearly stated this problem in relation to the resins and oils in hops. The cultivated varieties of hops are not a random sample. It is

not surprising that as a result of selection there are certain common characteristics within each of the three major groups of cultivated varieties, both in chemical and morphological features. These cultivated plants represent a very small fraction of the variation within the species. Also, within each group some common genetic background must occur, although their ancestry is very incompletely known. Working in the experimental fields at the Hops Research Station at Corvallis, Oregon, with the morphological complexes referred to earlier, the senior author found that among the many hundred of crosses there were many plants which would not fit easily into any of the three complexes suggested. The difficulties were much greater than had been observed among collections of wild hops throughout North America. Although this topic will be developed more fully in a later paper, it is clear that under natural conditions many of the potential hops types do not survive, and that there are strong forces limiting the variants within wild plants. For example, it was shown previously (Davis, 1957) that the wild hops of the midwestern United States are remarkably uniform, but that hops in the Rocky Mountains and New England are extremely variable. Only the American type has so far been found in the midwest, but apparent variants of Continental and English hops occur in the Western and Eastern parts of North America. Valuable as the morphological analysis has shown itself to be, we have had a considerable desire to find some other method of approach to determine if the heterogeneity in wild hops is real and related to that occurring among cultivated plants and not due to a weakness in the morphological classification itself. Cytological studies are being conducted, but because of the very limited information on chromosome types in wild American hops it is impossible to interpret the results at this time. Another alternative is offered by the humulone complex of substances which have been shown to be different in American and European cultivated hops. We have indicated that this method of analysis, which is firmly established in respect to the chemical procedure, still requires study before its biological and genetical value can

be established beyond any possible doubt, but that there is much information which justifies its use at present.

The wild hops of New England are particularly fascinating because in addition to their botanical variation they present an intriguing problem due to historical circumstances. It is not certain that hops are native to New England. Cultivation in the New England Colonies occurred so early (probably with English hops) that there is a question as to whether wild hops are exclusively escapes from cultivation or a mixture of wild and escaped plants. Asa Gray (1886) refers to wild hops scattered on banks of streams from Canada west to New Mexico, but he is not clear about New England where he notes that all cultivated plants are undoubtedly of European origin. Nuttall (1847) is certain that hops are native to the Rocky Mountains, but did not commit himself on New England. During Revolutionary days, New England and particularly the Connecticut River Valley, was the center of hops cultivation in the United States. There can be no doubt that English hops were grown here and had ample opportunity fifty to two hundred years ago to become established. Our previous analysis of New England hops, from herbarium material, has shown the leaf pubescence pattern typical of English hops to be by far the most common, but in at least some characteristics all three complexes are represented. As we gradually learn more about the hops now wild in New England we will know whether they are related to English hops only, or to Continental and American hops as well. Chemical analysis should contribute significantly to this picture.

EXPERIMENTAL PROCEDURE

The determination of the relative proportions of humulone, cohumulone and adhumulone in the alpha acids was first done by the technique of Verzele and Govaert (1955) by separating the humulone complex on a silica gel column, followed by the determination of the relative amount of each compound with a Beckman Du spectrophotometer, measuring absorbancy at 276 mμ. This procedure did permit a separation of the individual components, but it proved

very difficult to replicate the results quantitatively. A much more exact method, developed by Rigby, Sihto, and Bars (1960) involves extracting from the hops cones the alpha acids, precipitating them as a lead salt, pyrolyzing the salt to break off isobutyric, 2-methylbutyric, and isovaleric acid from the molecules of cohumulone, adhumulone and humulone respectively. Rigby has shown that this technique gives results very similar to that from a separation of the humulone complex by the much more time consuming countercurrent distribution technique. The fatty acids were then esterified with isopropyl alcohol, and the isopropyl esters separated on a chromatographic column (type R, Perkin-Elmer) at 100°C, and a helium gas flow rate of 120 ml/min, using a Perkin Elmer vapor fractometer, Model 154. The details of the extraction and preparation of the fatty acid esters are to be found in Methods of Analysis of Assoc. of Agric. Chemists (1945) and Rigby, Sihto, and Bars, (1960). The relative quantity of each component was determined by calculation of the areas under the curves for each acid, as recorded by the fractometer recorder, and the results reported as a percentage of the total alpha acid.

RESULTS

Several hops plants were collected from Massachusetts and Maine and their morphological and chemical characteristics determined. Other plants from Vermont, Maine and Massachusetts produced such a poor yield of cones that it was impossible to analyze the alpha acids and the morphological analysis of these plants has not been included in this study. For comparative purposes commercial samples of Idaho Seedless hops (a Late Clusters type) and cones from a plant of Late Clusters grown in Amherst are included. Late Clusters belongs to the American complex of hops. Table I contains the results of the chemical analysis. Three tests were run on each hop. Table 2 shows the results of the examination of morphological features. In each table, the sample is assigned to a "type" in the last column. The details of the morphological analysis is found elsewhere (Davis, 1957).

DISCUSSION

Comparison of the tables for morphological and chemical analysis shows that when the cohumulone scale is established as indicated, there is an exact correspondence between morphological and chemical types. The scale was established prior to this study, after comparing the work on cultivated varieties referred to above. It does not imply, for example, that all plants cultivated or wild, bearing the designation American, are of origin within North America. Such a conclusion could only be drawn at the completion of an extensive study. Rather, the terms American, Continental, and English were chosen because, of the wild and cultivated plants studied, these designations were the most descriptive of their center of distribution. It may be necessary to add additional designations for other parts of the world, or to establish several complexes for the large areas like North America. The more important goal is to establish a chemical analysis that will support morphological investigations. These preliminary results are very encouraging in this respect.

The hops from Biddeford, Maine were found growing in sand near the coast and could not have been in cultivation at any time. Those from Windsor, Massachusetts lined a fence near fields long deserted. They certainly had not been cultivated where they were growing, but might have escaped from cultivation in this area in recent times. The collection from Dalton, on the other hand, was of plants which had been used for brewing within the last 25 years.

Chemical analysis shows the Dalton hops to be strikingly similar to the cultivated Late Clusters, such as is grown in Idaho. The sources of its roots are unknown, but it is not unreasonable to suggest that they were obtained commercially from a supplier of cultivated hops, and not brought into cultivation from the wild state. The hops from Maine and Windsor, which may have been seedlings from cultivated plants of several generations ago are similar to English cultivated types. In morphological features they resemble the oldest herbarium material of New England hops.

With a continued examination of hops about which

detailed information on their history is available, we should be able to answer the question as to whether or not American type hops in New England are only of recent origin with as much certainty as is possible in this kind of taxonomic problem. Perhaps most important, these preliminary chemical tests confirm our previous observations of a morphological variation in New England wild hops which corresponds to the variation between cultivated hops throughout the world, and gives additional support to the use of morphological evaluation in tracing the evolution of hops.

— DEPT. OF BOTANY, UNIV. OF MASSACHUSETTS, AMHERST

TABLE I

CHEMICAL ANALYSIS

	Percentage of cohumulone	adhumulone	humulone	TYPE*
Idaho Seedless (1961 crop)	49.2	8.7	42.1	
	48.6	8.4	43.0	
	46.8	10.4	42.8	
average	48.2	9.2	42.6	American
Late Clusters (Amherst)	36.1	14.9	49.0	
	37.8	15.7	46.5	
	37.6	13.2	49.2	
average	37.2	14.6	48.2	American
Dalton, Mass.	46.0	10.5	43.5	
	47.9	8.6	43.5	
	45.6	12.2	42.2	
average	46.5	10.4	43.1	American
Windsor, Mass.	32.2	13.0	54.8	
	32.5	12.8	54.7	
	35.7	13.2	51.1	
average	33.5	13.0	53.5	English
Biddeford, Maine	29.6	13.1	57.3	
	24.9	12.9	62.2	
	24.8	12.8	62.4	
average	26.4	12.9	60.6	English

* The designation is based upon a scale for cohumulone of: Continental, 20-25%; English, 26-35%; American, 36% and over.

TABLE 2
MORPHOLOGICAL ANALYSIS

Origin of Hops	Cone		Leaf			Classification Type
	Bract scar on axis	Angularity of axis	Veins on Bract	Dentations on central lobe	Pubescence on lower surface	
Idaho Seedless (1961 crop)	Short	130°	11	36	On all veins & between veins	American
Late Clusters (Amherst)	Short	118°	11	44	On all veins & between veins	American
Dalton, Mass.	Short	110°	12	44	On all veins & between veins	American
Windsor, Mass.	Short	134°	7	24	On main veins only	English
Biddeford, Maine	Short	136°	7	24	On main veins only	English
"Typical"* American hops	Short	110°-130°	10-up	34 up	On all veins & between veins	American
"Typical"* English hops	Short	130°-150°	7-9	20-26	On main veins only	English

* Detailed descriptions of the American and English hops are to be found in the paper of Davis (1957).

LITERATURE CITED

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. 1945. Official and Tentative Methods of Analysis. Washington 4, D. C.

ALSTON, R. E. AND H. S. IRWIN. 1961. The comparative extent of variation of free amino acids and certain "secondary" substances among *Cassia* species. Am. Jour. Bot. **48**: 35-39.

DAVIS, E. L. 1957. Morphological complexes in hops (*Humulus lupulus* L.) with special reference to the American race. Ann. Missouri Bot. Gard. **44**: 271-294.

GRAY, ASA. 1886. American Druggist **15**: 111.

HOWARD, G. A. AND A. R. TATCHELL. 1956. Evaluation of hops. New approach to the detailed analysis of hop resins. J. Inst. Brew. **62**: 20-27.

NEVE, R. A. 1958. Sex chromosomes in the hop *Humulus lupulus*. Nature **181**: 1084-1085.

NEVE, R. A. AND E. W. WESTON. 1958. Resin and oil relationships in hops. J. Inst. Brew. **64**: 247-248.

NUTTALL, T. 1847. Descriptions of plants collected by William Gambel in the Rocky Mts. and upper California. Jour. Acad. Phil. N.S. **1**: 182.

RIGBY, F. L. 1956. Recent advances in the chemistry of hop constituents and their significance in brewing technology. Technical Proceeding Master Brew. Assoc. of Am. **69**: 9-16.

RIGBY, F. L., E. SIHTO AND A. BARS. 1960. A rapid method for the detailed analysis of the alpha acid fraction of hops by gas chromatography. J. Inst. Brew. **66**: 242-249.

TURNER, B. L. AND R. E. ALSTON. 1959. Segregation and recombination of chemical constituents in a hybrid swarm of *Baptisia laevicaulis* X *B. viridis* and their taxonomic implications. Am. Jour. Bot. **46**: 678-686.

VERZELE, M. AND F. GOVAERT. 1955. Analysis of the "Humulone Complex" by a chromatographic partition procedure. Wallerstein Lab. Commun. **18**: 181-190.

CHROMOSOME NUMBERS IN THE COMPOSITAE. VI. ADDITIONAL MEXICAN AND GUATEMALAN SPECIES.

B. L. TURNER, M. POWELL AND R. M. KING

The present contribution is essentially a continuation of several papers, the latest of which (Turner *et al.*, 1961a)